Cytogenetics and genetic engineering

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History: The Central Dogma

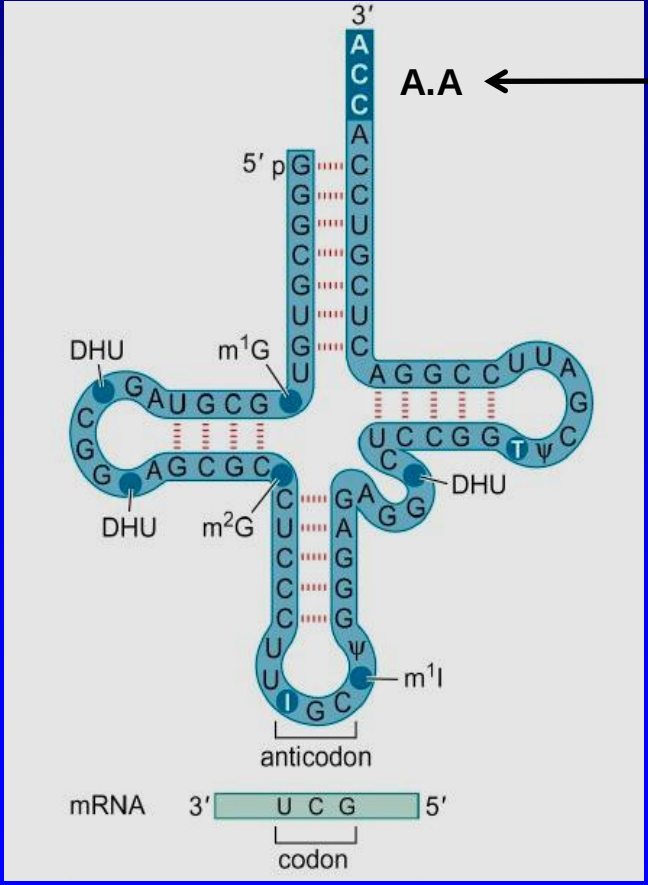
- The double helical nature of DNA theoretically precluded it as being the “simplest” or “most efficient” template for protein synthesis. (To get protein synthesis from DNA you would have to separate the strands to make single stranded DNA before protein synthesis could begin.)
- However, the single stranded nature of RNA, along with RNA’s chemically similar nature to DNA, did indicate it could serve as the template for protein synthesis.
- In 1956 Francis Crick hypothesized a **central dogma** for the flow of genetic information:

- In this dogma, DNA is the template for self replication.
- DNA is also the template for the synthesis of RNA (**transcription**).
- Protein synthesis is then driven from the RNA template in the process of **translation**.

Continue: Central Dogma

- In 1957 Kornberg demonstrated that a single enzyme (DNA polymerase I) was capable of catalyzing the synthesis of a new DNA strand utilizing the DNA and dATP, dGTP, dCTP, and dTTP.
- DNA Polymerase I links the nucleotides to the growing DNA strand by a 3’ to 5’ phosphodiester bond and ONLY in the presence of DNA.
- In 1955, Francis Crick proposed that prior to their incorporation into proteins, amino acids are first attached to a specific adaptor molecule called transfer RNA (tRNA) by enzymes called aminoacyl synthetases.

Continue: Central Dogma



The tRNA has a 3’-end where the amino acid is attached and an “anticodon” loop that recognizes the RNA template.

Continue: Central Dogma

History: Ribosomal and mRNA

Next question, what form of RNA is the template to make protein?

- Because ribosomal RNA comprised about 85% of cellular RNA, it was believed to be the template for protein synthesis.

  However, analysis of the composition of ribosomal RNA ruled out this possibility.

- In 1960, a new class of RNA was discovered in phage studies.

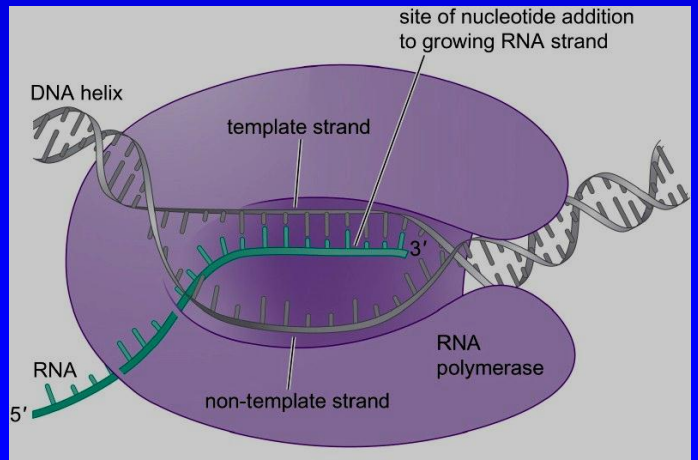
  Because this phage RNA carried the information from DNA to the sites of protein synthesis, it was called **messenger RNA (mRNA)**.

- mRNA comprises only about 4% of cellular RNA - has large variations in length and nucleotide composition.

Continue: Central Dogma

History: RNA Polymerase

- Around the same time as the discovery of mRNA, Jerard Hurwitz and Sam B. Weiss were isolating the enzyme that synthesized RNA from DNA.
- **RNA polymerase** - functions only in the presence of DNA and uses the nucleotides ATP, GTP, CTP, and UTP as precursors.
- During transcription, only one of the two strands of DNA is used as the template (the anti-sense strand). Synthesis is always in a fixed direction (5' to 3').
- Once synthesized, the mRNA is transported from the nucleus (where DNA is located) to the cytoplasm (where the ribosomes are located) for translation into protein.



Eukaryotic RNA Polymerases

Unlike bacteria (which have only one RNA polymerase), eukaryotes have three: RNA Pol I, RNA Pol II, and RNA Pol III.

Table 10.1 Roles of Eukaryotic RNA Polymerases

RNA Polymerase	Cellular RNAs Synthesized	Mature RNA (Vertebrate)
I	Large rRNA precursor	28S, 18S, and 5.8S rRNAs
II	hnRNAs	mRNAs
	snRNAs	snRNAs
III	5S rRNA precursor	5S rRNA
	tRNA precursors	tRNAs
	U6 snRNA (precursor?)	U6 snRNA
	7SL RNA (precursor?)	7SL RNA
	7SK RNA (precursor?)	7SK RNA

RNA Pol II and the synthesis of mRNA (protein encoding genes) will be the primary focus of this lecture. The other polymerases will be briefly discussed.

Transcription and Replication: Similar……

- The process of transcription is biochemically and enzymatically very similar to the process of replication.
- Both processes involve the use of enzymes to synthesize a strand of nucleic acids from a DNA template.
- The synthesis occurs in the 5'- to 3'- direction.
- However transcription differs from replication in several very important ways:

Transcription and Replication: …….yet Different

1. Transcription is performed by the enzyme **RNA polymerase**. RNA polymerase does not require a primer to initiate the polymerization reaction. It can initiate synthesis de novo, directed by very specific DNA sequences .
2. Unlike replication, which reproduces both strands of DNA, only one strand of the DNA acts as the template for transcription.
3. The RNA product does not remain base paired to the DNA template. It is displaced by RNA polymerase after a few bases allowing multiple **transcripts** (or RNA products) to be produced from the same gene at the same time.
4. Transcription is less accurate (1 mistake every 10,000 nucleotides added) than replication (1 mistake every 10,000,000 nucleotides added).
5. Transcription selectively copies only certain parts of the genome (known as **genes**) making several hundred if not several thousand copies of each gene while replication must replicate the entire genome every cell doubling making only a single copy
6. The process of transcription can be highly regulated depending on which genes must be transcribed, when they must be transcribed, and how much of each gene product is needed.

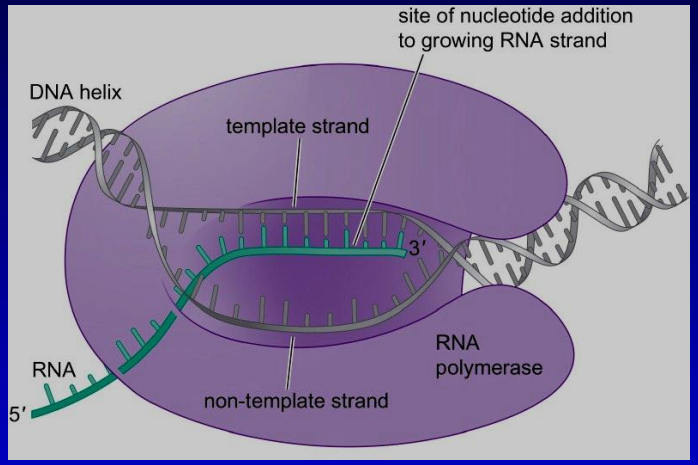
Transcription vs. Replication: Why Error Repair Isn't as Important

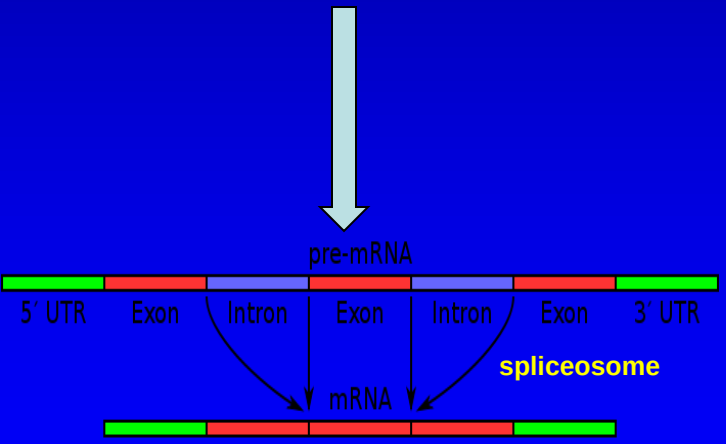
- transcription is less accurate than replication (1 mistake every 10,000 nucleotides added for transcription vs. 1 mistake every 10,000,000 nucleotides added for replication).
- transcription lacks the extensive proofreading that is present in replication.
- Transcription generates multiple products.
- These products are usually transient and short lived.
- Mistake would generate single, mutated protein out of many normal proteins of the same type. (Not much impact.)
- Mistake in replication - creates an error in the genome, which is permanent and potentially catastrophic.
- Therefore, replication must be highly accurate to prevent this

Summary

	Replication	Transcription
Direction of synthesis	5’----------3’	5’----------3’
Bases	dTTP, dGTP, dATP and dCTP	UTP, GTP, ATP and CTP
Template	Both strands of DNA	Template strand (3’--------5’)
Copies	The whole geome	Genes only
No. of copies	2 daughter strands of DNA	Many transcripts of the same gene
End product	DNA	mRNA
Enzymes	DNA polymerase I	RNA polymerase II
Accuracy	More accurate (1 mistake every 10,000,000)	Less accurate (1 mistake every 10,000)

Transcription: Overview





Transcription proceeds through a series of three distinct stages:

1) **Initiation:** RNA polymerase binds to the **promoter** region of DNA. (The promoter is the DNA sequence that instructs the transcriptional complex to bind DNA at the beginning of a gene to be transcribed.) Once bound, the promoter-polymerase complex undergoes structural changes.

2) **Elongation:** Once the RNA polymerase has synthesized a small stretch of RNA, it undergoes additional conformational changes to grip it to the DNA tighter. This is the active process of RNA synthesis.

3) **Termination:** Once the polymerase reaches the end of the gene, it stops transcription and releases the RNA product.

Transcription Initiation: Overview

Initiation can be further broken down into several distinct steps:

a) The polymerase binds to the promoter region in order to form a **closed complex** with DNA strand.

b) Once bound, the DNA closed complex undergoes a transition to the **open complex**, in which approximately 14 bp of DNA is melted (or separated) to form a "bubble".

- This process frees the template strand
- The first two ribonucleotides (of the RNA strand) are brought in and aligned and covalently joined.
- The polymerase begins to move along the DNA, opening the double helix ahead of it until the first ten or so ribonucleotides are incorporated (a very inefficient process with many short aborted RNA transcripts released)

Continue: Transcription Initiation

C) Once the first 10 or so ribonucleotides are incorporated, the enzyme has "escaped" the promoter and forms a **stable ternary complex**, which contains enzyme, DNA template and growing RNA transcript.

- Transcription elongation, termination and RNA processing are initiated by several factors bound to RNA polymerase II C-terminal domain (CTD)

RNA Processing

RNA processing involves three distinct steps:

1. 5'-capping of the RNA
2. RNA splicing
3. Polyadenylation

RNA Processing: 5’-Capping

- 5’-capping involves the addition of a methylated guanine joined to the RNA transcript in a novel 5’-5’ linkage.
- Capping occurs when the transcript is about 20 - 40 bases long (or at the transition from initiation to elongation).
- Capping occurs in three steps:

1. Removal of a 5’ phosphate from the RNA
2. Addition of the GMP
3. Methylation of the added GMP

RNA Processing: Polyadenylation

- Polymerase carries two elongation factors that can recognize and bind to the **poly-A signal sequence** (5'-A A U A A A-3'), in order to add a long AAAA tail, after which it cleaves the RNA product.

RNA Processing: RNA Splicing

- This process is carried out by specific enzyme called spliceosome, which is a complex of snRNA and protein subunits. This enzyme remove introns from a transcribed pre-mRNA segment to form a mature mRNA.

  This process is generally referred to as splicing.

mRNA Translation

Translation Initiation (General)

Three events for the successful initiation of translation .

1. The ribosome must be recruited to the mRNA.
2. A charged tRNA must be placed in the P-site of the ribosome.
3. The ribosome must be positioned over the start codon.

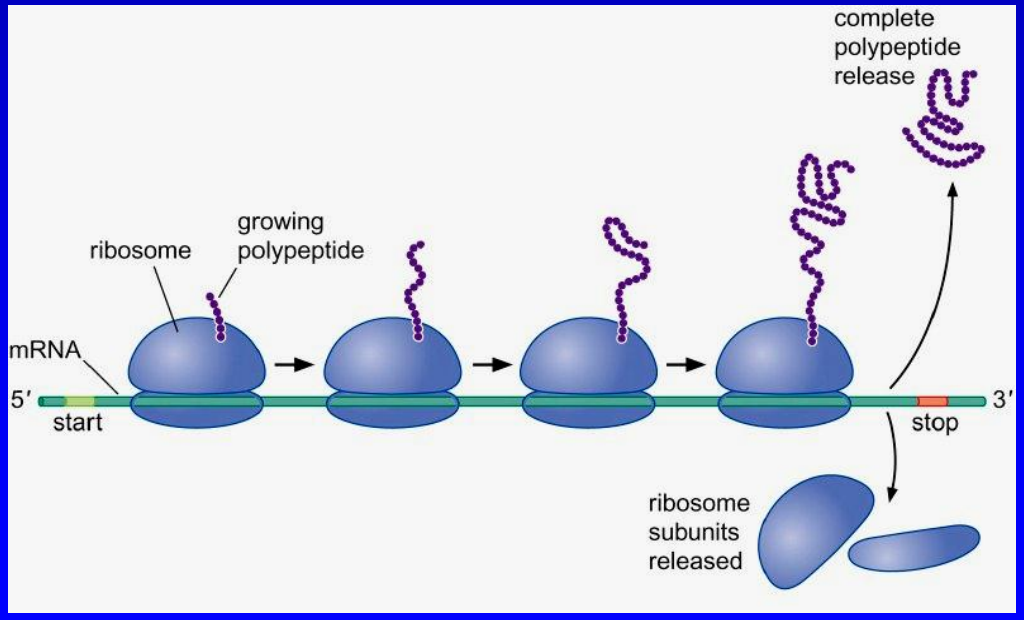
Eukaryotic mRNA: The Kozak Sequence

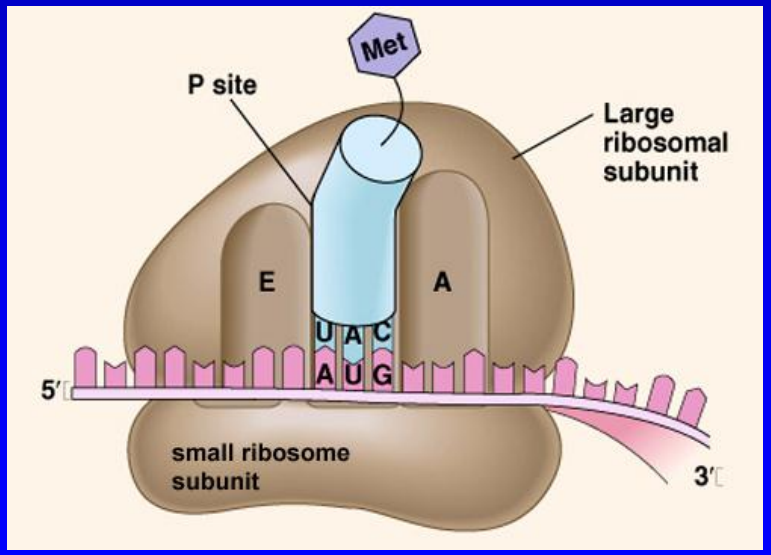
- It was discovered, that in 5 - 10% of eukaryotic genes, the first "encountered" AUG was not the initiating AUG. If the first encountered AUG is not always used, then **what determines an initiation AUG?**
- An examination of the DNA sequences surrounding the known initiation AUGs identified a common consensus sequence **(Kozak sequence)** that identifies the starting codon.

- This sequence defines the initiation codon for the beginning of eukaryotic translation (underlined).
- The arrows indicate the most critical bases.

Translation Elongation

- Three basic parts of the translation process:
  1. The correct charged tRNA must be delivered to the open A-site.
  2. A peptide bond must be formed between the growing chain in the P-site and the new amino acid in the A-site.
  3. The resulting peptidyl-tRNA in the A-site must be translocated to the P-site with a simultaneous translocation of the empty tRNA from the P-site to the E-site.
- These processes are assisted by two **elongation factors**.





Translation Termination: Release Factors

- Three codons exist (UAA, UGA and UAG) that code for termination- that is, they do not incorporate an amino acid but instead terminate translation.
- Long believed that there is a chain-terminating tRNA that would recognize these codons.
- Instead - termination is accomplished by proteins called **release factors (RF)**.
- Two classes:

1. **Class I Release Factors**: recognize the stop codon and trigger the release of the peptide chain.
2. **Class II Release Factors**: stimulate the release of the Class I release factor after the peptide chain has been liberated.

END